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On the early onset of thymineless death occurring after exposure to ultraviolet light

GALLANT AND SUSKIND[1] have presented data indicating that death due to lack of thymine (thymineless death) and irradiation with ultraviolet light act on the same sites in *Escherichia coli* strain B-3. The basis for their interpretation is the reduced time of onset of thymineless death following ultraviolet-light irradiation together with the reduced multiplicity of ultraviolet-light inactivation curves of organisms that are prestarved for thymine. This report confirms their observations and shows some additional features of the cooperative action of ultraviolet light and lack of thymine in this system.

E. coli strain B-3 (obtained from Dr. F. FORRO) was maintained on minimal agar slants containing 1 % glucose and 5 μg/ml thymidine (Nutritional Biochemicals Corp.). The formula for the minimal medium was modified from ROBERTS *et al.*[2] and contained NH_4Cl, 0.02 %; Na_2HPO_4, 0.6 %; KH_2PO_4, 0.3 %; NaCl, 0.3 %; $MgCl_2 \cdot 6\,H_2O$, 0.01 %; Na_2SO_4, 0.012 %. The pH was 6.9. The minimal agar contained 1.5 % Bacto-Agar (Difco).

To prepare cultures for irradiation, the organisms were subcultured twice in minimal medium containing 1 mg/ml glucose and 5 μg/ml thymidine. (The response of the organisms in this system is essentially the same with the free base or the nucleoside[3].) The low concentration of glucose limited growth to about 10^9 organisms per ml. For irradiation, 18-h cultures were diluted with the minimal salt solution to 10^7 organisms per ml. 10-ml samples of this dilution were exposed in 10-cm petri dishes to 2537-Å radiation from a low-pressure mercury arc (Mineralight) at a dose rate of about 5 ergs/mm^2/sec. 1-ml samples of the irradiated suspension were transferred to flasks containing 99 ml of the minimal salt solution with thymidine or glucose added at the times indicated in the figures. Incubation was done in a water bath at 37° with continuous agitation. At various times, samples were taken, suitably diluted with minimal salts, and plated on minimal agar.

Photoreactivation was accomplished by exposing the plated organisms to four 40-W fluorescent lamps in a white enamel reflector for 15 min and at a distance of 2 inches from the top of the covered plastic petri dishes. All manipulations other than the exposure to photoreactivation light were done in a photographic darkroom under dim light. Ultraviolet-light irradiation and photoreactivation were done at room temperature.

Fig. 1 shows the early onset of thymineless death as reported by GALLANT AND SUSKIND[1]. The exponential death of the organisms does not continue but shows a period following the initial drop during which the rate of loss of viability is much less. Also, the slope of the initial drop appears slightly steeper than that of thymineless death in non-irradiated organisms. These two observations are also apparent in the data of GALLANT AND SUSKIND[1], but were not discussed by them. Following the period of reduction in rate of loss of viability, exponential death again resumes with a slope that appears to parallel that of thymineless death occurring in unirradiated organisms.

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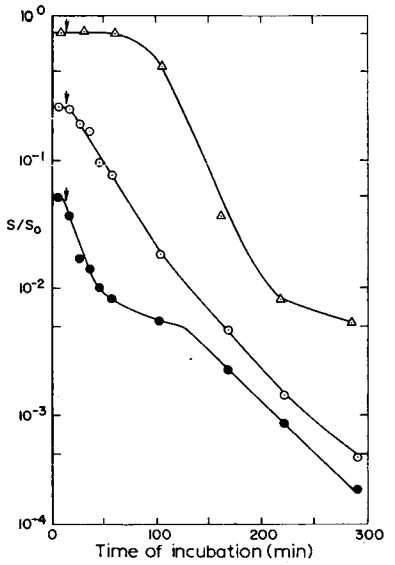

Fig. 1. Early onset of thymineless death and photoreactivation of ultraviolet-light-irradiated *E. coli* B-3. △–△, non-irradiated control; ●–●, ultraviolet-light irradiated; ○–○, ultraviolet-light irradiated and photoreactivated. The ultraviolet-light inactivating dose was about 500 ergs/per mm^2. The arrows indicate the time of addition of glucose to the cultures to a concentration of 2 mg/ml.

If the ultraviolet-light-irradiated organisms are subjected to photoreactivation (Fig. 1) the early loss of viability is still apparent, but the slope of this loss is about one-half that of the organisms held in the dark. Since when thymidine is present the initial drop in viability is eliminated altogether, these data indicate that effects produced by ultraviolet light causing an early loss of viability can be reversed either by photoreactivation or by incubation with thymidine. The curve showing the loss of viability of photoreactivated organisms (Fig. 1) shows no sharp break after the initial drop as in the case of the non-photoreactivated ones. If one considers that the ability to be photoreactivated is lost as the cells continue to metabolize[4] and introduces a correction for this then the break in the curve reappears (Fig. 2). This correction for loss of photoreactivation is based on cultures containing thymidine and may not be entirely valid for cultures not having it.

Ultraviolet light produces a variety of rather specific photo-products involving the bases in the nucleic acids[5]. Of these the thymine dimer is subject to photoreactivation[6]. Our results indicate that ultraviolet-light lesions contributing to the early onset of thymineless death may be partially photoreactivated. The most obvious deduction is that thymine dimer is involved in producing early thymineless death. If this is not true, then one must conclude that there are other ultraviolet-light lesions which are also subject to photoreactivation.

The significance of the delay in continued death following the initial drop in viability is not clear. In any case, our results indicate heterogeneity in the effects of ultraviolet light under the conditions of these experiments; different types of damage may be involved in producing early death and delay of continued death. Comparison of the photoreactivation curves in Fig. 2 with those of a non-photoreactivated culture

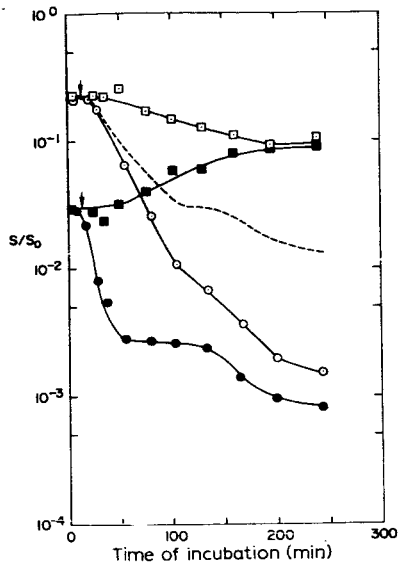

Fig. 2. Comparison of photoreactivation of ultraviolet-light-irradiated *E. coli* B-3 when incubated with and without thymidine. ⊡–⊡, with thymidine and photoreactivated; ■–■, with thymidine and not photoreactivated; ⊙–⊙, without thymidine and photoreactivated; ●–●, without thymidine and not photoreactivated. The arrows indicate the time of addition of glucose to the cultures. The dashed line is the calculated level of photoreactivation if the ability to be photoreactivated was not lost with time.

shows that early death may be reversed by either photoreactivation or the presence of thymidine. However, later death can only be prevented by thymidine, and the lag before onset is unaffected by photoreactivation. These results indicate that this heterogeneity may be a result of different kinds of ultraviolet photoproducts.

Work on this problem is continuing in order to clarify the role of various photoproducts in this system.

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